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	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND		
☐	L1	chlamyd$.ti,ab,clm.	2588
☐	L2	L1 and salmone$.ti,ab,clm.	360
☐	L3	L2 and (mammal$ or animal$ or eukaryote or eukaryotic or eucaryote or eucaryotic or cho or human or fibroblast).ti,ab,clm.	253
☐	L4	(momp or mompa or momp-a or (membrane near2 protein)).ti,ab,clm.	4958
☐	L5	L4 and l3	7
☐	L6	5770714.pn.	2
☐	L7	l1 and l4	194
☐	L8	L7 and (mammal$ or animal$ or eukaryote or eukaryotic or eucaryote or eucaryotic or cho or human or fibroblast).ti,ab,clm.	99
☐	L9	(brunham or murdin).in.	139
☐	L10	L9 and chlamyd$	109
	DB=USPT; PLUR=YES; OP=AND		
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☐	L12	(brunham or murdin).in.	31
☐	L13	L12 and chlamyd$	20

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C12Q001/68 G01N033/53 G01N033/543 G01N033/566 G01N033/569 C12N001/21 C12N015/09 C12P021/02 C12R001:01 C12R001:01 C12R001:01.

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US-PAT-NO: 6838085
DOCUMENT-IDENTIFIER: US 6838085 B2

TITLE: DNA immunization against Chlamydia infection

DATE-ISSUED: January 4, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Brunham; Robert C.	Winnipeg			CA

US-CL-CURRENT: 424/263.1; 424/185.1, 435/252.3, 435/471, 530/350, 530/389.5, 530/412, 536/22.1, 536/23.1, 536/23.7

CLAIMS:

What I claim is:

1. A non-replicating vector, comprising: a nucleotide sequence encoding a region comprising at least one of the conserved domains 2, 3 and 5 of a major outer membrane protein of a strain of Chlamydia, and a promoter sequence operatively coupled to said nucleotide sequence for expression of said at least one conserved domain in a host.

2. The vector of claim 1 wherein said nucleotide sequence encoding the conserved domain 2 and/or 3 further includes a nucleotide sequence encoding a variable domain of the major outer membrane protein immediately downstream of the conserved domain.

3. The vector of claim 1 wherein said nucleotide sequence encodes the conserved domain 5 of the outer membrane protein.

4. The vector of claim 1 wherein said promoter sequence is the cytomegalovirus promoter.

5. The vector of claim 1 wherein said non-replicating vector comprises plasmid pcDNA3 containing said promoter sequence and into wherein said nucleotide sequence is inserted in operative position to said promoter sequence.

6. The vector of claim 5 wherein said strain of Chlamydia is a strain producing chlamydial infectious of the lung.

7. The vector of claim 5 wherein said strain of Chlamydia is a strain of Chlamydia trachomatis.

L8: Entry 32 of 99 File: USPT Feb 3, 2004

US-PAT-NO: 6686339
DOCUMENT-IDENTIFIER: US 6686339 B1

TITLE: Nucleic acid molecules encoding inclusion membrane protein C of Chlamydia

DATE-ISSUED: February 3, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Murdin; Andrew D.	Newmarket			CA
Dunn; Pamela L.	Mississauga			CA
Oomen; Raymond P.	Schomberg			CA

US-CL-CURRENT: 514/44; 424/93.2, 435/320.1, 536/23.1, 536/23.2, 536/24.1

CLAIMS:

What we claim is:

1. An expression cassette comprising an isolated nucleic acid molecule placed under the control of elements required for expression of said nucleic acid molecule, said isolated nucleic acid molecule comprising a polynucleotide sequence encoding an amino acid sequence selected from the group consisting of: (a) an amino acid sequence as set forth in SEQ ID NO: 3; and (b) a fragment of the sequence in (a), said fragment comprising at least 12 amino acids and being capable of inducing an immune response against Chlamydia.

2. The expression cassette according to claim 1 wherein, in (b), said fragment comprises at least 20 amino acids.

3. The expression cassette according to claim 1 wherein, in (b), said fragment comprises at least 50 amino acids.

4. The expression cassette according to claim 1 wherein, in (b), said fragment comprises at least 75 amino acids.

5. The expression cassette according to claim 1 wherein, in (b), said fragment comprises at least 100 amino acids.

6. The expression cassette according to claim 1 wherein, in (b), said amino acid sequence retains the specific antigenicity of SEQ ID NO: 3.

7. The expression cassette according to claim 1, said nucleic acid molecule comprising a polynucleotide sequence encoding the amino acid sequence as set forth in SEQ ID NO: 3.

8. The expression cassette according to claim 1, wherein said polynucleotide sequence comprises the sequence set forth in SEQ ID NO: 1 or 2.

9. An expression vector comprising the expression cassette of claim 1.

10. A vaccine vector comprising an isolated nucleic acid molecule placed under the control of elements required for expression of said isolated nucleic acid molecule, said nucleic acid molecule comprising a polynucleotide sequence encoding an amino acid sequence selected from the group consisting of: (a) an amino acid sequence as set forth in SEQ ID NO: 3; and (b) a fragment of the sequence in (a), said fragment comprising at least 12 amino acids and being capable of inducing an immune response against Chlamydia.

11. The vaccine vector according to claim 10 wherein, in (b), said fragment comprises at least 20 amino acids.

12. The vaccine vector according to claim 10 wherein, in (b), said fragment comprises at least 50 amino acids.

13. The vaccine vector according to claim 10 wherein, in (b), said fragment comprises at least 75 amino acids.

14. The vaccine vector according to claim 10 wherein, in (b), said fragment comprises at least 100 amino acids.

15. The vaccine vector according to claim 10 wherein, in (b), said amino acid sequence retains the specific antigenicity of SEQ ID NO: 3.

16. The vaccine vector according to claim 10, said nucleic acid molecule comprising a polynucleotide sequence encoding the amino acid sequence as set forth in SEQ ID NO: 3.

17. The vaccine vector according to claim 10, wherein said polynucleotide sequence comprises the sequence set forth in SEQ ID NO: 1 or 2.

18. The vaccine vector according to claim 10 wherein the elements required for expression include a promoter.

19. The ~~vaccine vector~~ according to claim 18 wherein the promoter is a cytomegalovirus promoter.

20. The vaccine vector according to claim 19, which is a plasmid vector.

21. The vaccine vector of claim 20 wherein said plasmid vector has the identifying characteristics of plasmid pCAI115, as shown in FIG. 3.

22. An imunogenic composition comprising an isolated nucleic acid molecule comprising a polynucleotide sequence encoding an amino acid sequence selected from the group consisting of: (a) an amino acid sequence as set forth in SEQ ID NO: 3; and (b) a fragment of the sequence in (a), said fragment comprising at least 12 amino acids and being capable of inducing an immune response against Chlamydia.

23. An immunogenic composition comprising a vaccine vector according to claim 10.

24. An immunogenic composition comprising a vaccine vector according to claim 11.

25. An immunogenic composition comprising a vaccine vector according to claim

12.

26. An immunogenic composition comprising a vaccine vector according to claim 13.

27. An immunogenic composition comprising a vaccine vector according to claim 14.

28. An immunogenic composition comprising a vaccine vector according to claim 15.

29. An immunogenic composition comprising a vaccine vector according to claim 16.

30. An immunogenic composition comprising a vaccine vector according to claim 17.

31. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 23.

32. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 24.

33. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 25.

34. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 26.

35. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 27.

36. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 28.

37. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 29.

38. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 30.

L8: Entry 42 of 99 File: USPT Aug 29, 2000

US-PAT-NO: 6110898
DOCUMENT-IDENTIFIER: US 6110898 A

TITLE: DNA vaccines for eliciting a mucosal immune response

DATE-ISSUED: August 29, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Malone; Robert W.	Baltimore	MD		
Malone; Jill G.	Baltimore	MD		

US-CL-CURRENT: 514/44; 424/204.1, 424/234.1, 424/256.1, 424/93.1, 435/455, 435/6, 435/69.1, 435/91.1

CLAIMS:

What is claimed is:

1. A method for inducing a mucosal immune response in a host comprising locally administering to said host an antigen-encoding polynucleotide preparation, whereby administration of said polynucleotide preparation is specifically targeted to mucosal inductor sites.

2. The method of claim 1, wherein said host is a mammal.

3. The method of claim 2, wherein said mammal is a human.

4. The method of claim 1, wherein said antigen-encoding polynucleotide preparation is a viral vector.

5. The method of claim 4, wherein said viral vector contains heterologous regions which encode for epitopic regions of at least one immunogenic protein.

6. The method of claim 5, wherein said immunogenic protein is encoded by a virus selected from the group consisting of Human Papalloma Virus, Herpes Simplex Virus, and Human Immunodeficiency Virus.

7. The method of claim 6, wherein said virus is Human Papalloma Virus.

8. The method of claim 5, wherein said immunogenic protein is the Human Papilloma Virus major viral capsid protein L1.

9. The method of claim 6, wherein said virus is Herpes Simplex Virus.

10. The method of claim 5, wherein said immunogenic protein is the Herpes Simplex Virus immediate early protein ICP 27.

11. The method of claim 6, wherein said virus is Human Immunodeficiency Virus.

12. The method of claim 5, wherein said immunogenic protein is the all or part of the Human Immunodefieiency Virus envelope, gag, nef, or tat proteins.

13. The method of claim 5, wherein said viral vector includes a recombinant alphavirus vector system.

14. The method of claim 1, wherein said antigen-encoding polynucleotide preparation is derived from a prokaryote.

15. The method of claim 14, wherein said prokaryote contains heterologous genetic regions which encode for epitopic regions of at least one immunogenic protein.

16. The method of claim 14, wherein said prokaryote is selected from the group consisting of Helicobacter Pylorii and Chlamydia trachomatis.

17. The method of claim 15, wherein said immunogenic protein is all or part of the Helicobacter Pylorii urease protein.

18. The method of claim 15, wherin said immunogenic protein is all or part of the Chlamydia trachomatis major outer membrane protein.

19. The method of claim 1, wherein said mucosal inductor sites are selected from the group consisting of Waldeyer's ring, Peyer's patches, gut-associated lymphoid tissues, bronchial associated lymphoid tissues, nasal-associated lymphoid tissues, genital-associated lymphoid tissues, and tonsils.

20. A method for polynucleotide delivery to the mucosal tissue of a host comprising locally administering to said host an antigen-encoding polynucleotide preparation, whereby administration of said polynucleotide preparation is specifically targeted to mucosal inductor sites.

21. The method of claim 20, wherein said host is a mammal.

22. The method of claim 21, wherein said mammal is a human.

23. The method of claim 20, wherein said antigen-encoding polynucleotide preparation is a viral vector.

24. The method of claim 23, wherein said viral vector contains heterologous regions which encode for epitopic regions of at least one immunogenic protein.

25. The method of claim 24, wherein said immunogenic protein is encoded by a virus selected from the group consisting of Human Papalloma Virus, Herpes Simplex Virus, and Human Immunodeficiency Virus.

26. The method of claim 25, wherein said virus is Human Papalloma Virus.

27. The method of claim 24, wherein said immunogenic protein is the Human Papilloma Virus major viral capsid protein L1.

28. The method of claim 25, wherein said virus is Herpes Simplex Virus.

29. The method of claim 24, wherein said immunogenic protein is the Herpes

Simplex Virus immediate early protein ICP 27.

30. The method of claim 25, wherein said virus is Human Immunodeficiency Virus.

31. The method of claim 24, wherein said immunogenic protein is the all or part of the Human Immunodefieiency Virus envelope, gag, nef, or tat proteins.

32. The method of claim 1, wherein said antigen-encoding polynucleotide preparation is derived from a prokaryote.

33. The method of claim 32, wherein said prokaryote contains heterologous genetic regions which encode for epitopic regions of at least one immunogenic protein.

34. The method of claim 32, wherein said prokaryote is selected from the group consisting of Helicobacter Pylorii and Chlamydia trachomatis.

35. The method of claim 33, wherein said immunogenic protein is all or part of the Helicobacter Pylorii urease protein.

36. The method of claim 33, wherin said immunogenic protein is all or part of the Chlamydia trachomatis major outer membrane protein.

37. The method of claim 23, wherein said viral vector includes a recombinant alphavirus vector system.

38. The method of claim 20, wherein said mucosal inductor sites are selected from the group consisting of Waldeyer's ring, Peyer's patches, gut-associated lymphoid tissues, bronchial associated lymphoid tissues, nasal-associated lymphoid tissues, genital-associated lymphoid tissues, and tonsils.

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Leu Thr Ala Glu Ala Arg Leu Ile Asn Glu Arg Ala Ala His Val Ser
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Gly Gln Phe Arg Phe
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What I claim is:

1. A non-replicating vector, comprising:

a nucleotide sequence encoding a region comprising at least one of the conserved domains 2, 3 and 5 of a major outer membrane protein of a strain of *Chlamydia*, and

a promoter sequence operatively coupled to said nucleotide sequence for expression of said at least one conserved domain in a host.

2. The vector of claim 1 wherein said nucleotide sequence encoding the conserved domain 2 and/or 3 further includes a nucleotide sequence encoding a variable domain of the major outer membrane protein immediately downstream of the conserved domain.

3. The vector of claim 1 wherein said nucleotide sequence encodes the conserved domain 5 of the outer membrane protein.

4. The vector of claim 1 wherein said promoter sequence is the cytomegalovirus promoter.

5. The vector of claim 1 wherein said non-replicating vector comprises plasmid pcDNA3 containing said promoter sequence and into wherein said nucleotide sequence is inserted in operative position to said promoter sequence.

6. The vector of claim 5 wherein said strain of *Chlamydia* is a strain producing chlamydial infectious of the lung.

7. The vector of claim 5 wherein said strain of *Chlamydia* is a strain of *Chlamydia trachomatis*.

* * * * *

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We claim:

1. An immunogenic composition, comprising:

a first plasmid vector comprising:

a first nucleotide sequence encoding a major outer membrane protein (MOMP) of a strain of *Chlamydia pneumoniae*, said first nucleotide sequence being selected from the group consisting of SEQ ID Nos: 12, 13, and 14 or encoding a MOMP having an amino acid sequence selected from the group consisting of SEQ ID Nos: 15 and 16, and

a first promoter sequence operatively coupled to said first nucleotide sequence for expression of said MOMP in a host;

a second plasmid vector comprising:

a second nucleotide sequence encoding a 76 kDa protein of a strain of *Chlamydia pneumoniae*, said second nucleotide sequence being selected from the group consisting of SEQ ID Nos: 1, 2, 3 and 4, and a second promoter sequence operatively coupled to said second nucleotide sequence for expression of said 76 kDa protein in a host; and

a pharmaceutically-acceptable carrier therefor.

2. The immunogenic composition of claim 1 wherein the first promoter is a cytomegalovirus promoter.

3. The immunogenic composition of claim 1 wherein said second nucleotide sequence is 76 kDa protein gene sequence encoding a protein having a molecular size of about 35 kDa and having SEQ ID No: 7.

4. The immunogenic composition of claim 1 wherein said second nucleotide sequence is 76 kDa protein gene sequence encoding a protein having a molecular size of about 60 kDa and having SEQ ID No: 8 or 9.

5. The immunogenic composition of claim 1 wherein said second promoter is a cytomegalovirus promoter.

6. The immunogenic composition of claim 1 wherein said first plasmid vector is pCAMOMP and said second plasmid vector is pCA76 kDa.

7. The immunogenic composition of claim 1 wherein said first and second vectors are present in amounts such that upon administration of the composition to the host, the protective effect of the first vector is not adversely affected by the second vector and the protective effect of the second vector is not adversely affected by the first vector.

8. The immunogenic composition of claim 1 wherein said first and second vectors are present in amounts such that an enhanced protective effect is achieved in comparison to the individual vectors alone.

* * * * *

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What is claimed is:

1. An isolated nucleic acid molecule which encodes the polypeptide SEQ ID NO:2.

2. An isolated nucleic acid molecule comprising the nucleic acid sequence SEQ ID No: 1.

3. An isolated nucleic acid molecule which is anti-sense to the nucleic acid molecule of claim 1.

4. An isolated nucleic acid molecule which encodes a fusion protein, said fusion protein comprising the polypeptide encoded by the nucleic acid molecule of claim 1 and a second polypeptide.

5. The nucleic acid molecule of claim 4 wherein the second polypeptide is a heterologous signal peptide.

6. The nucleic acid molecule of claim 4 wherein the second polypeptide has adjuvant activity.

7. The nucleic acid molecule of claim 4, operably linked to one or more expression control sequences.

8. A vaccine vector comprising the nucleic acid sequence selected from any one of:

(i) SEQ ID No: 1; or

(ii) a nucleic acid sequence which encodes the polypeptide of SEQ ID NO:2;

wherein the nucleic acid sequence is capable of being expressed.

9. The vaccine vector of claim 8 comprising a hybrid gene, wherein the hybrid gene encodes a fusion polypeptide, wherein the fusion polypeptide comprises the polypeptide of

SEQ ID No: 2; and

heterologous-polypeptide;

wherein the hybrid gene is capable of being expressed.

10. The vaccine vector of claim 9 wherein the second polypeptide is a heterologous signal peptide.

11. The vaccine vector of claim 9 wherein the second polypeptide has adjuvant activity.

12. The vaccine vector of claim 8 wherein the nucleic acid is operably linked to one or more expression control sequences.

13. The vaccine vector of claim 8 wherein the polypeptide-encoding nucleic acid is the first nucleic acid, and wherein the vaccine vector further comprises a second nucleic acid encoding an additional polypeptide which enhances the immune response to the polypeptide expressed by said first nucleic acid.

14. The vaccine vector of claim 13 wherein the additional polypeptide is a *Chlamydia* polypeptide.

15. A pharmaceutical composition comprising the nucleic acid according to claim 1 and a pharmaceutically acceptable carrier.

16. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent, and a nucleic acid molecule which encodes the polypeptide of SEQ ID NO:2; wherein the nucleic acid is capable of being expressed.

17. A unicellular host transformed with the nucleic acid molecule of claim 7.

18. A method for preventing or treating *Chlamydia pneumoniae* infection comprising administering to a patient an effective amount of:

(a) the nucleic acid according to claim 1;

(b) a vaccine vector wherein the vaccine vector comprises the nucleic acid according to claim 1;

(c) a pharmaceutical composition comprising the nucleic acid according to claim 1 and a pharmaceutically acceptable carrier; or

(d) the polypeptide encoded by the nucleic acid according to claim 1 in the reading frame set forth in SEQ ID NO:2.

19. The vaccine vector according to claim 8 wherein the vaccine vector is expression plasmid pCAI764 as shown in FIG. 3.

* * * * *

-continued

<211> LENGTH: 35
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<213> ORGANISM: Chlamydia trachomatis

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<400> SEQUENCE: 2

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I claim:

1. An immunogenic composition for intranasal or intramuscular administration to a host for the generation in the host of a protective immune response to a major outer membrane protein (MOMP) of a strain of *Chlamydia trachornatis* or *Chlamydia pneumoniae*, comprising a non-replicating vector suitable for DNA vaccine use, comprising:

a nucleotide sequence encoding said MOMP or an N-terminal fragment of approximately half full-length MOMP, and

a cyomegalovirus promoter sequence operatively coupled to said nucleotide sequence for expression of said MOMP in the host; and

a pharmaceutically-acceptable carrier therefor.

2. The immunogenic composition of claim 1 wherein said nucleotide sequence encodes full-length MOMP.

3. The immunogenic composition of claim 1 wherein said strain of Chlamydia is a strain of *Chlamydia trachomatis*.

4. The immunogenic composition of claim 3 wherein said non-replicating vector comprises plasmid pcDNA3 containing said promoter sequence and into which said nucleotide sequence is inserted in operative relation to said promoter sequence.

5. The immunogenic composition of claim 1 wherein said immune response is predominantly a cellular immune response.

6. The immunogenic composition of claim 1 wherein said nucleotide sequence encodes said MOMP which stimulates a recall immune response following exposure to wild-type Chlamydia.

7. A method of immunizing a host against disease caused by infection with a strain of *Chlamydia trachomatis* or *Chlamydia pneunioniae*, which comprises administering to said host intranasally or intramuscularly an effective amount of a non-replicating vector comprising:

a nucleotide sequence encoding a major outer membrane protein (MOMP) of a strain of *Chlamydia trachomatis* or *Chlamydia pneumoniae* or an N-terminal fragment of approximately half the full-length MOMP, and

a promoter sequence operatively coupled to said nucleotide sequence for expression of said MOMP in the host.

8. The method of claim 7 wherein said nucleotide sequence encodes full-length MOMP.

9. The method of claim 7 wherein said nucleotide sequence encodes an N-terminal fragment of approximately half of full length MOMP.

10. The method of claim 7 wherein said promoter sequence is a cytomegalovirus promoter.

11. The method of claim 7 wherein said strain of Chlamydia is a strain of *Chlamydia trachomatis*.

12. The method of claim 7 wherein said non-replicating vector comprises plasmid pcDNA3 containing said promoter into which said nucleotide sequence is inserted in operative relation to said promoter sequence.

13. The method of claim 7 wherein said immune response is predominantly a cellular immune response.

14. The method of claim 7 wherein said nucleotide sequence encodes said MOMP which stimulates a recall immune response following exposure to wild-type Chlamydia.

15. The method of claim 7 wherein said non-replicating vector is administered intranasally.

16. A method of using a gene encoding a major outer membrane protein (MOMP) of a strain of *Chlamydia trachomatis* or *Chlamycha pneumoniae* or an N-terminal fragment of approximately half of the full-length MOMP, which comprises:

isolating said gene,

operatively linking said gene to at least one control sequence to produce a non-replicating vector, said control sequence directing expression of said MOMP or fragment thereof when introduced into a host to produce an immune response to said MOMP or fragment thereof, and

introducing said vector into a host intranasally or intramuscularly.

17. The method of claim 16 wherein said gene encoding MOMP encodes full length MOMP.

18. The method of claim 16 wherein said gene encoding MOMP encodes an N-terminal fragment of approximately half of full-length MOMP.

19. The method of claim 16 wherein said control sequence is a cytomegalovirus promoter.

20. The method of claim 16 wherein said strain of Chlamydia is a strain of *Chlamydia trachomatis*.

21. The method of claim 16 wherein said non-replicating vector comprises plasmid pcDNA3 containing said control sequence into which said gene encoding MOMP is inserted in operative relation to said control sequence.

22. The method of claim 16 wherein said immune response is predominantly a cellular immune response.

23. The method of claim 16 wherein said gene encodes said MOMP which stimulates a recall immune response following exposure to wild-type Chlamydia.

24. The method of claim 16 wherein said vector is introduced into said host intranasally.

* * * * *

What we claim is:

1. An isolated and purified nucleic acid molecule comprising a polynucleotide sequence encoding an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence as set forth in SEQ ID NO:3; and

(b) a fragment of the sequence in (a), said fragment comprising at least 50 amino acids and being capable of inducing an immune response against Chlamydia;

or the complementary polynucleotide sequence thereto.

2. The nucleic acid molecule according to claim 1 wherein, in (b), said fragment comprises at least 75 amino acids.

3. The nucleic acid molecule according to claim 1 wherein, in (b), said fragment comprises at least 100 amino acids.

4. The nucleic acid molecule according to claim 1 wherein, in (b), said amino acid sequence retains the specific antigenicity of SEQ ID NO:3.

5. The nucleic acid molecule of claim 1, said nucleic acid molecule comprising a polynucleotide sequence encoding the amino acid sequence as set forth in SEQ ID No:3, or the complementary polynucleotide sequence thereto.

6. The nucleic acid molecule of claim 1, wherein said polynucleotide sequence is the sequence set forth in SEQ ID NO:1 or 2, or the complementary polynucleotide sequence thereto.

7. An expression cassette comprising a polynucleotide sequence of claim 1 placed under the control of elements required for expression of the polynucleotide sequence.

8. An expression vector comprising the expression cassette of claim 7.

9. A vaccine vector comprising the nucleic acid of claim 1 placed under the control of elements required for expression.

10. The vector of claim 9 which is a plasmid vector.

11. The vector of claim 10 wherein said plasmid vector is plasmid pCAI327.

12. An immunogenic composition comprising a vaccine vector according to claim 9.

13. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 12.

14. An immunogenic composition comprising a nucleic acid molecule according to claim 1.

15. An antibody that specifically binds to a polypeptide selected from the group consisting of:

(a) an amino acid sequence as set forth in SEQ ID No:3; and

(b) a fragment of the sequence in (a), said fragment comprising at least 50 amino acids and being capable of inducing an immune response against Chlamydia.

16. A primer pair for PCR amplification of genomic nucleic acid encoding a POMP91A of a strain of *Chlamydia pneumoniae* from the genome of the strain of *Chlamydia pneumoniae* which comprises:

5' primer: 5'-ATAAGAATGCGGCCGCCACCATGAAGCAGATGGTTCTTTGGG-3' (SEQ ID No:4) and

3' primer: 5'-GCGCCGGTACCGGAAACTAAGGGAGAGGCCTGCATG-3' (SEQ ID No:5).

* * * * *

What we claim is:

1. An expression cassette comprising an isolated nucleic acid molecule placed under the control of elements required for expression of said nucleic acid molecule, said isolated nucleic acid molecule comprising a polynucleotide sequence encoding an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence as set forth in SEQ ID NO: 3; and

(b) a fragment of the sequence in (a), said fragment comprising at least 12 amino acids and being capable of inducing an immune response against Chlamydia.

2. The expression cassette according to claim 1 wherein, in (b), said fragment comprises at least 20 amino acids.

3. The expression cassette according to claim 1 wherein, in (b), said fragment comprises at least 50 amino acids.

4. The expression cassette according to claim 1 wherein, in (b), said fragment comprises at least 75 amino acids.

5. The expression cassette according to claim 1 wherein, in (b), said fragment comprises at least 100 amino acids.

6. The expression cassette according to claim 1 wherein, in (b), said amino acid sequence retains the specific antigenicity of SEQ ID NO: 3.

7. The expression cassette according to claim 1, said nucleic acid molecule comprising a polynucleotide sequence encoding the amino acid sequence as set forth in SEQ ID NO: 3.

8. The expression cassette according to claim 1, wherein said polynucleotide sequence comprises the sequence set forth in SEQ ID NO: 1 or 2.

9. An expression vector comprising the expression cassette of claim 1.

10. A vaccine vector comprising an isolated nucleic acid molecule placed under the control of elements required for expression of said isolated nucleic acid molecule, said nucleic acid molecule comprising a polynucleotide sequence encoding an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence as set forth in SEQ ID NO: 3; and

(b) a fragment of the sequence in (a), said fragment comprising at least 12 amino acids and being capable of inducing an immune response against Chlamydia.

11. The vaccine vector according to claim 10 wherein, in (b), said fragment comprises at least 20 amino acids.

12. The vaccine vector according to claim 10 wherein, in (b), said fragment comprises at least 50 amino acids.

13. The vaccine vector according to claim 10 wherein, in (b), said fragment comprises at least 75 amino acids.

14. The vaccine vector according to claim 10 wherein, in (b), said fragment comprises at least 100 amino acids.

15. The vaccine vector according to claim 10 wherein, in (b), said amino acid sequence retains the specific antigenicity of SEQ ID NO: 3.

16. The vaccine vector according to claim 10, said nucleic acid molecule comprising a polynucleotide sequence encoding the amino acid sequence as set forth in SEQ ID NO: 3.

17. The vaccine vector according to claim 10, wherein said polynucleotide sequence comprises the sequence set forth in SEQ ID NO: 1 or 2.

18. The vaccine vector according to claim 10 wherein the elements required for expression include a promoter.

19. The vaccine vector according to claim 18 wherein the promoter is a cytomegalovirus promoter.

20. The vaccine vector according to claim 19, which is a plasmid vector.

21. The vaccine vector of claim 20 wherein said plasmid vector has the identifying characteristics of plasmid pCAI115, as shown in FIG. 3.

22. An imunogenic composition comprising an isolated nucleic acid molecule comprising a polynucleotide sequence encoding an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence as set forth in SEQ ID NO: 3; and

(b) a fragment of the sequence in (a), said fragment comprising at least 12 amino acids and being capable of inducing an immune response against Chlamydia.

23. An immunogenic composition comprising a vaccine vector according to claim 10.

24. An immunogenic composition comprising a vaccine vector according to claim 11.

25. An immunogenic composition comprising a vaccine vector according to claim 12.

26. An immunogenic composition comprising a vaccine vector according to claim 13.

27. An immunogenic composition comprising a vaccine vector according to claim 14.

28. An immunogenic composition comprising a vaccine vector according to claim 15.

29. An immunogenic composition comprising a vaccine vector according to claim 16.

30. An immunogenic composition comprising a vaccine vector according to claim 17.

31. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 23.

32. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 24.

33. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 25.

34. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 26.

35. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 27.

36. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 28.

37. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 29.

38. A method for inducing an immune response against Chlamydia, comprising administering to a host an effective amount of an immunogenic composition according to claim 30.

* * * * *

SEQUENCE LISTING

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<210> SEQ ID NO 1
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<212> TYPE: DNA
<213> ORGANISM: Chlamydia trachomatis

<400> SEQUENCE: 2

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What we claim is:

1. A method of immunizing a host, which comprises:

initially administering to the host an attenuated bacteria harbouring a vector comprising a nucleic acid molecule encoding a major outer membrane protein (MOMP) of a strain of Chlamydia and a promoter sequence operatively coupled to said nucleic acid molecule for expression of said MOMP of a strain of Chiamydia in cells of the host but not in said attenuated bacteria, and

subsequently administering to the host a purified major outer membrane protein (MOMP) of a strain of Chlamydia.

2. The method of claim 1 wherein said strain of Chlamydia is a strain of *Chlamydia pneumoniae.*

3. The method of claim 1 wherein said strain of Chiamydia is a strain of *Chlamydia trachomatis.*

4. The method of claim 1 wherein said attenuated bacteria is an attenuated strain of Salmonella.

5. The method of claim 1 wherein said vector is a plasmid vector.

6. The method of claim 1 wherein said MOMP of a strain of Chlamydia in said subsequent administration step is administered incorporated into an immunostimulating complex (ISCOM).

7. The method of claim 6 wherein said strain of Chlamydia is a strain of *Chlamydia pneumoniae.*

8. The method of claim 6 wherein said strain of Chlamydia is a strain of *Chlamydia trachomatis.*

9. The method of claim 1 wherein said initial administration step is effected to mucosal surfaces.

10. The method of claim 9 wherein said initial administration step is effected by intranasal administration and said subsequent administration step is effected by intramuscular administration.

11. A method of immunizing a host, which comprises:

initially administering to the host an attenuated bacterial harbouring a vector comprising a nucleic acid molecule encoding a major outer membrane protein (MOMP) of a strain of Chlamydia and a promoter which is a cytomegalovirus promoter operatively coupled to said nucleic acid molecule for expression of said MOMP of a strain of Chlamydia in cells of the host, and

subsequently administering to the host a purified major outer membrane protein (MOMP) of a strain of Chlamydia.

12. A method of immunizing a host, which comprises:

initially administering to the host an attenuated bacteria harbouring a plasmid vector which is pcDNA3/MOMP as seen in FIG. 5, and

subsequently administering to the host a purified major outer membrane protein (MOMP) of a strain of Chlamydia.

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```
Lys Pro Thr Phe Thr Lys Thr Tyr Leu Ser Gly Phe Phe Lys Lys Lys
        355                 360                 365

Arg Thr Tyr Thr Asn Pro Asp Thr Asn Leu His Gly Glu Thr Arg Pro
    370                 375                 380

Ile Ile Asp Thr Asp Ile Tyr Asp Lys Val Met Pro Met Arg Ile Pro
385                 390                 395                 400

Val Val Pro Leu Ile Lys Ala Val Ile Thr Lys Asn Phe Asp Leu Ala
                405                 410                 415

Asn Glu Leu Gly Phe Leu Glu Val Cys Gly Glu Asp Phe Ala Leu Pro
            420                 425                 430

Thr Leu Ile Asp Pro Ser Lys Thr Glu Met Leu Thr Ile Val Lys Glu
        435                 440                 445
```

What is claimed is:

1. An isolated polypeptide from a strain of Chiamydia that has at least 90% identity to SEQ ID NO:4, wherein said isolated polypeptide, when administered in an immunogenically-effective amount to a mammal, induces an immune response by said mammal against said strain of Chiamydia.

2. The polypeptide of claim 1, wherein said polypeptide has the sequence of SEQ ID NO:2.

3. A polypeptide comprising the polypeptide of claim 1 linked to a fusion polypeptide.

4. The polypeptide of claim 3, wherein the fusion polypeptide is a signal peptide.

5. The polypeptide of claim 3, wherein the fusion polypeptide comprises a heterologous polypeptide having adjuvant activity.

* * * * *

of type-, subspecies-, species-, and genus-reactive antibody binding domains on the major outer membrane protein of *Chlamydia trachomatis*. Mol. Microbiol. 2: 673–679.

33. Conlan, J. W., S. Ferris, I. N. Clarke, and M. E. Ward. 1990. Isolation of recombinant fragments of the major outer membrane protein of *Chlamydia trachomatis*: their potential as subunit vaccines. J. Gen. Microbial. 136: 2013–2020.

34. Morrison, R. P., D. S. Manning, and H. D. Caldwell. 1992. Immunology of *Chlamydia trachomatis* infections. p. 57–84 In T. C. Quinn (ed) Sexually transmitted diseases. Raven Press Ltd., NY.

35. Kersten, G. F. A. and Crommelin, D. J. A. 1995. Liposomes and ISCOMs as vaccine formulations. Biochimica et Biophysica Acta 1241 (1995) 117–138.

36. Morein, B., Fossum, C., Lovgren, K. and Hoglund, S., 1990. The iscom—a modern approach to vaccines. seminars in Virology, Vol. 1, 1990: pp 49–55.

37. Mowat & Reid, 1992. Preparation of Immune Stimulating Complexes (ISCOMs) as Adjuvants. Current Protocols in Immunology 1992, Supplement 4: 2.11.1 to 2.11.12.

What we claim is:

1. A method of producing an outer membrane antigen extract of a strain of Chlamydia, which comprises:

detergent extracting elementary bodies of said strain of Chlamydia in the presence of a reducing agent to solubilize cytoplasmic material away from outer membrane material;

separating said solubilized cytoplasmic material from the outer membrane materials;

detergent extracting said outer membrane material using at least two non-ionic detergents in the presence of a reducing agent to solubilize outer membrane antigens; and

separating said solubilized outer membrane antigens from residual unextracted membrane-associated material to provide said outer membrane antigen extract.

2. The method of claim 1 wherein said at least two non-ionic detergents comprise a N-methylglucamide non-ionic detergent and a glucopyranoside non-ionic detergent.

3. The method of claim 2 wherein said N-methylglucamide non-ionic detergent is selected from the group consisting of heptanoyl-, octanoyl-, nonanoyl- and decanoyl-N-methylglucamide.

4. The method of claim 3 wherein said glucopyranoside non-ionic detergent is selected from the group consisting of n-hexyl-β-D, n-heptyl-β-D, n-octyl-α-D-, n-octyl-β-D, n-nonyl-β-D, n-decyl-α-D- and n-decyl-α-D-glucopyranoside.

5. The method of claim 4 wherein said two non-ionic detergents are employed in a weight-ratio from about 1:10 to about 10:1.

* * * * *

-continued

```
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gggcactgta gttttgctct tagtatgatc aaacagtttc ttaatcagcg aacagagaaa   1020

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```

What I claim is:

1. A pcDNA3 plasmid vector comprising:

a nucleotide sequence encoding a serine-threonine kinase (STK) of a strain of *Chlamydia trachomatis*, and consisting of SEQ ID No: 1, and

a promoter sequence operatively coupled to said nucleotide sequence for expression of said STK in a host to which the vector is administered, said promoter sequence being the human cytomegalovirus major intermediate-early promoter-enhancer region.

* * * * *

-continued

<212> TYPE: DNA
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<400> SEQUENCE: 5

gcgccggatc cgagaagccg gtagaggcgt g 31

What we claim is:

1. An isolated and purified nucleic acid molecule encoding an inclusion membrane protein C of a strain of Chlamydia having a polynucleotide sequence selected from the group consisting of:

(a) a polynucleotide sequence having SEQ ID Nos: 1 or 2 or the complementary polynucleotide sequence thereto, and

(b) a polynucleotide sequence encoding an amino acid sequence having SEQ ID No: 3.

2. The nucleic acid molecule of claim 1 which is retrieved from the strain of Chlamydia by PCR amplification of genomic bacterial DNA using synthetic oligonucleotide primers having a nucleotide sequence complementary to 5' and 3' ends of the encoding domain.

3. The nucleic acid molecule of claim 2 wherein each said primers consist of about 10 to 40 nucleotides.

4. The nucleic acid molecule of claim 2 wherein each said primers consist of about 15 to 25 nucleotides.

5. The nucleic acid molecule of claim 3 wherein each said primers contains at least about 40% of the nucleotides which are C and G nucleotides to ensure efficient hybridization.

6. The nucleic acid molecule of claim 4 wherein each said primers contains at least about 50% of the nucleotides which are C and G nucleotides to ensure efficient hybridization.

* * * * *

membrane protein of *Chlamydia trachomatis*. Infect. Immun. 31:1161–1176.

20. Bavoil, p., Ohlin, A. and Schachter, J., 1984. Role of Disulfide Bonding in Outer Membrane Structure and permeability in *Chlamydia trachomatis*. Infect. Immun., 44: 479–485

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25. Yuan, Y., Y. -X. Zhang, N. G. Watkins, and H. D. Caldwell. 1989. Nucleotide and deduced amino acid sequences for the four variable domains of the major outer membrane proteins of the 15 *Chlamydia trachomatis* serovars. Infect. Immun. 57:104–1049.

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30. Baehr, W., Y. -X. Zhang, T. Joseph, H. Su, F. E. Nano, K. D. E. Everett and H. D. Caldwell. 1988. Mapping antigenic domains expressed by *Chlamydia trachomatis* major outer membrane protein genes. proc. Natl. Acad. Sci. USA, 85:4000–4004.

31. Stephens., R. S., E. A. Wagar and G. K. Schoolnik. 1988. High-resolution mapping of serovar-specific and common antigenic determinants of the major outer membrane protein of *Chlamydia trachomatis*. J. Exp. Med. 167:817–831.

32. Conlan, J. W., I. N. Clarke and M. E. Ward. 1988. Epitope mapping with solid-phase peptides: identification of type-, subspecies-, species-, and genus-reactive antibody binding domains on the major outer membrane protein of *Chlamydia trachomatis*. Mol. Microbiol. 2: 673–679.

33. Conlan, J. W., S. Ferris, I. N. Clarke, and M. E. Ward. 1990. Isolation of recombinant fragments of the major outer membrane protein of *Chlamydia trachomatis:* their potential as subunit vaccines. J. Gen. Microbial. 136: 2013–2020.

34. Morrison, R. P., D. S. Manning, and H. D. Caldwell. 1992. Immunology of *Chlamydia trachomatis* infections. p. 57–84 In T. C. Quinn (ed) Sexually transmitted diseases. Raven press Ltd., N.Y.

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36. Morein, B., Fossum, C., Lovgren, K. and Hoglund, S., 1990. The iscom—a modern approach to vaccines. seminars in Virology, Vol. 1, 1990: pp 49–55.

37. Mowat & Reid, 1992. Preparation of Immune Stimulating Complexes (ISCOMs) as Adjuvants. Current protocols in Immunology 1992, Supplement 4: 2.11.1 to 2.11.12.

What we claim is:

1. An immunogenic composition, comprising an outer membrane antigen extract (MAE) of a strain of Chlamydia and an immunostimulating complex (ISCOM), said MAE comprising a major outer membrane protein (MOMP) of the strain of Chlamydia free from the heat shock protein HSP60 of the strain of Chlamydia.

2. The composition of claim 1 wherein said MOMP is in an oligomeric form.

3. The immunogenic composition of claim 2 wherein said oligomeric form of MOMP has a molecular weight of from about 45 to about 125 kDa.

4. The immunogenic composition of claim 1, wherein the outer membrane antigen extract is incorporated into immunostimulatory complexes (ISCOMs).

5. A method of protecting a host against disease caused by a strain of Chlamydia, comprising administering to said host an effective amount of the immunogenic composition of claim 1.

6. The method of claim 5 wherein said administration is to a mucosal surface of said host to produce a mucosal immune response.

7. The method of claim 6 wherein said administration to said mucosal surface is by intranasal administration to produce a vaginal tract immune response.

8. The immunogenic composition of claim 2 wherein said MOMP is complexed with at least one antigen of the strain of Chlamydia.

9. The immunogenic composition of claim 8 wherein said complex has a molecular weight of about 45 to about 125 kDa.

* * * * *

-continued

```
Asp Val Leu Gly Tyr Val Ala His Ile Tyr A sn Glu Asp Thr Gln Lys
            340                 345                 350

Thr Leu Ala Ser Ile Thr Ser Trp Cys Gln P ro Val Ile Leu Ile Phe
        355                 360                 365

Leu Gly Gly Leu Ile Gly Val Ile Met Leu A la Ile Leu Ile Pro Leu
    370                 375                 380

Thr Ser Asn Ile Gln Thr Leu
385                 390


<210> SEQ ID NO 3
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Chlamydia pneumoniae

<400> SEQUENCE: 3

ataagaatgc ggccgccacc atgcctcgat atcggtata                          39


<210> SEQ ID NO 4
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Chlamydia pneumoniae

<400> SEQUENCE: 4

gcgccggatc cctaatgttt ggatattg                                      28
```

What is claimed is:

1. An isolated polypeptide having a sequence that is at least 75% identical to SEQ ID NO: 2, wherein said isolated polypeptide, when administered in an immunogenically-effective amount to a mammal, elicits the production of antibodies against said polypeptide and induces an immune response by said mammal.

2. The polypeptide of claim 1, wherein said polypeptide has the sequence of SEQ ID NO: 2.

3. A polypeptide comprising the polypeptide of claim 1 linked to a fusion polypeptide.

4. The polypeptide of claim 3, wherein the fusion polypeptide is a signal peptide.

5. The polypeptide of claim 3, wherein the fusion polypeptide comprises a heterologous polypeptide having adjuvant activity.

6. A pharmaceutical composition, comprising the immunogenically-effective amount of the polypeptide of claim 1 and pharmaceutically acceptable diluent.

7. The pharmaceutical composition of claim 6, further comprising an adjuvant.

* * * * *

-continued

```
Tyr Gln Val Ile Val His Gly Gly Pro Phe V al Val Asn Met Thr Lys
        195                 200                 205

Lys His Tyr Ala Trp Val Val Glu Gly Ile L eu Asn Arg Leu Pro Lys
    210                 215                 220

Gln Phe Phe Val Lys Cys Ser Val Val Asp T rp Asn Thr Phe Val Pro
225                 230                 235                 240

Ser Glu Thr Ser Thr Thr Glu Lys Ala Ala T hr Asn Ala Met Lys Tyr
                245                 250                 255

Lys Tyr Cys Val Trp Gln Trp Leu Val Gly L ys His Ser Gln Val Pro
            260                 265                 270

Trp Ile Asn Gly Gln Lys Lys Pro Leu Tyr L eu Tyr Gly Ala Phe Leu
        275                 280                 285

Met Asn Pro Leu Ala Lys Ala Thr Lys Thr T hr Leu Asn Gly Lys Glu
    290                 295                 300

Asn Leu Ala Trp Phe Ile Gly Gly Thr Leu G ly Gly Leu Arg Lys Ala
305                 310                 315                 320

Gly Asp Trp Ser Ala Thr Val Arg Tyr Glu T yr Val Glu Ala Leu Ser
                325                 330                 335

Val Pro Glu Ile Asp Val Ser Gly Ile Gly A rg Gly Asn Leu Leu Lys
            340                 345                 350

Phe Trp Phe Ala Gln Ala Ile Ala Ala Asn T yr Asp Pro Lys Glu Ala
        355                 360                 365

Asn Gly Phe Thr Asn Tyr Lys Gly Phe Ser A la Leu Tyr Met Tyr Gly
    370                 375                 380

Ile Thr Asp Ser Leu Ser Phe Arg Ala Tyr G ly Ala Tyr Ser Lys Pro
385                 390                 395                 400

Ala Asn Asp Lys Leu Gly Ser Asp Phe Thr P he Arg Lys Phe Asp Leu
                405                 410                 415

Gly Ile Ile Ser Ala Phe
            420
```

<210> SEQ ID NO 3
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Chlamydia pneumoniae

<400> SEQUENCE: 3

ataagaatgc ggccgccacc atgtcaggat acgctgaact tcc 43

<210> SEQ ID NO 4
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Chlamydia pneumoniae

<400> SEQUENCE: 4

cggggtaccg aaacgctgaa attataccta 30

What is claimed is:

1. An isolated polypeptide having a sequence that is at least 75% identical to SEQ ID NO: 2, wherein said isolated polypeptide, when administered in an immunogenically-effective amount to a mammal, elicits the production of antibodies against said polypeptide and induces an immune response by said mammal.

2. The polypeptide of claim 1 wherein said polypeptide has the sequence of SEQ ID NO: 2.

3. A polypeptide comprising the polypeptide of claim 1 linked to a fusion polypeptide.

4. The polypeptide of claim 3, wherein the fusion polypeptide is a signal peptide.

5. The polypeptide of claim 3, wherein the fusion polypeptide comprises a heterologous polypeptide having adjuvant activity.

6. A pharmaceutical composition, comprising the immunogenically-effective amount of the polypeptide of claim 1 and pharmaceutically acceptable diluent.

7. The pharmaceutical composition of claim 6, further comprising an adjuvant.

* * * * *

-continued

<210> SEQ ID NO 16
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Chlamydia trachomatis

<400> SEQUENCE: 16

ggggatccgc caccatgctg cctgtgggga atcct 35

<210> SEQ ID NO 17
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Chlamydia trachomatis

<400> SEQUENCE: 17

ggggctcgag ctattaacgg aactgagc 28

What I claim is:

1. An immunogenic composition for in vivo administration to a host for the generation in the host of a protective immune response to a major outer membrane protein (MOMP) of a strain of Chlamydia, comprising a non-replicating vector comprising:

a nucleotide sequence encoding a region consisting of at least one of the conserved domains 2, 3 and 5 of a major outer membrane protein (MOMP) of a strain of Chlamydia, and

a promoter sequence operatively coupled to said nucleotide sequence for expression of said at least one conserved domain in the host; and

a pharmaceutically-acceptable carrier therefor.

2. An immunogenic composition for in vivo administration to a host for the generation in the host of a protective immune response to a major outer membrane protein (MOMP) of a strain of Chlamydia, comprising a non-replicating vector comprising:

a nucleotide sequence encoding a region consisting of at least one of the conserved domains 2 and 3 of a major outer membrane protein (MOMP) of a strain of Chlamydia and further consisting of a nucleotide sequence encoding a variable domain of the major outer membrane protein immediately downstream of said conserved domain, and

a promoter sequence operatively coupled to said nucleotide sequence for expression of said at least one conserved domain and said variable domain in the host; and

a pharmaceutically-acceptable carrier therefor.

3. The immunogenic composition of claim 1 wherein said nucleotide sequence encodes the conserved domain 5 of a major outer membrane protein of a strain of Chlamydia.

4. The immunogenic composition of claim 1 or 2 wherein said promoter sequence is a cytomegalovirus promoter.

5. The immunogenic composition of claim 1 or 2 wherein said strain of Chlamydia is a strain producing chlamydial infections of the lung.

6. The composition of claim 1 or 2 wherein said immune response is predominantly a cellular immune response.

7. The composition of claim 1 or 2 wherein said nucleotide sequence encodes a MOMP which stimulates a recall immune response following exposure to wild-type Chlamydia.

8. The immunogenic composition of claim 1 or 2 wherein said strain of Chlamydia is a strain of *Chlamydia trachomatis*.

9. The immunogenic composition of claim 8 wherein said non-replicating vector comprises plasmid pcDNA3 containing said promoter sequence and into which said nucleotide sequence is inserted in operative relation to said promoter sequence.

10. A vaccine for protection of a host against disease caused by infection with a strain of Chlamydia, produced by a method, which comprises:

isolating a nucleotide sequence selected from the group consisting of:

(i) a nucleotide sequence encoding a region consisting of at least one of the conserved domains 2, 3 and 5 of a major outer membrane protein (MOMP) of a strain of Chlamydia, and

(ii) a nucleotide sequence encoding a region consisting of at least one of the conserved domains 2 and 3 of a major outer membrane protein (MOMP) of a strain of Chlamydia and further consisting of a nucleotide sequence coding of a nucleotide sequence encoding a variable domain of the major outer membrane protein immediately downstream of the conserved domain,

operatively linking said nucleotide sequence to at least one control sequence to produce a non-replicating vector, the control sequence directing expression of said at least one conserved domain and said variable domain when introduced to a host to produce an immune response thereto, and

formulating said vector as a vaccine for in vivo administration to a host.

11. A non-replicating vector, comprising:

a nucleotide sequence encoding a region consisting of at least one of the conserved domains 2, 3 and 5 of a major outer membrane protein (MOMP) of a strain of Chlamydia, and

a promoter sequence operatively coupled to said nucleotide sequence for expression of said at least one conserved domain in a host.

12. A non-replicating vector, comprising:

a nucleotide sequence encoding a region consisting of at least one of the conserved domains 2 and 3 of a major outer membrane protein (MOMP) of a strain of

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What we claim is:

1. An immunogenic composition for in vivo administration to a host for the generation in the host of a protective immune response to a major outer membrane protein (MOMP) of a strain of Chiamydia, comprising:

a non-replicating plasmid vector comprising:

a nucleotide sequence encoding said MOMP or a fragment of said MOMP that generates a MOMP-specific immune response, and

a cptomeqalovirus promoter sequence operatively coupled to said nucleotide sequence for expression of said MOMP in the host; and

a pharmaceutically-acceptable carrier therefor.

2. The composition of claim 1 wherein said nucleotide sequence encodes full-length MOMP.

3. The immunogenic composition of claim 1 wherein said nucleotide sequence encodes an N-terminal fragment of said MOMP of approximately half the size of full-length MOMP.

4. The immunogenic composition of claim 1 wherein said strain of Chlamydia is a strain producing chlamydial infections of the lung.

5. The immunogenic composition of claim 1 wherein said strain of Chlamydia is a strain of *Chlamydia trachomatis*.

6. The immunogenic composition of claim 5 wherein said non-replicating plasmid vector is plasmid pcDNA3 containing said promoter sequence and into which said nucleotide sequence is inserted in operative relation to said promoter sequence.

7. The composition of claim 1 wherein said immune response is predominantly a cellular immune response.

8. The composition of claim 1 wherein said nucleotide sequence encodes said MOMP which stimulates a recall immune response following exposure to wild-type Chlamydia.

9. A vaccine produced by a method which comprises:

isolating a nucleotide sequence encoding a major outer membrane protein (MOMP) of a strain of Chiamydia or a fragment of said MOMP that generates a MOMP-specific immune response,

operatively linking said nucleotide sequence to at least one control sequence including a cytomegalovirus promoter to produce a non-replicating plasmid vector, the control sequence directing expression of said MOMP when introduced to a host to produce an immune response to said MOMP, and

formulating said vector as a vaccine for in vivo administration to a host.

* * * * *

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United States Patent [19]

Agabian et al.

[11] Patent Number: 5,770,714

[45] Date of Patent: Jun. 23, 1998

[54] **CHLAMYDIA MAJOR OUTER MEMBRANE PROTEIN**

[75] Inventors: **Nina Agabian**, San Francisco; **Richard Stephens**, Oakland, both of Calif.; **Cho-Chou Kuo**, Seattle, Wash.; **Guy Mullenbach**, Oakland, Calif.

[73] Assignees: **Washington Research Foundation**, Seattle, Wash.; **Chiron Corporation**, Emeryville, Calif.

[21] Appl. No.: **466,814**

[22] Filed: **Jun. 6, 1995**

Related U.S. Application Data

[62] Division of Ser. No. 144,095, Oct. 28, 1993, abandoned, which is a continuation of Ser. No. 691,639, Apr. 25, 1991, abandoned, which is a continuation of Ser. No. 818,523, Jan. 13, 1986, abandoned, which is a continuation-in-part of Ser. No. 692,001, Jan. 14, 1985, abandoned.

[51] **Int. Cl.**[6] **C07H 21/02**; C07H 21/04; C07K 5/00; C07K 13/00

[52] **U.S. Cl.** **536/23.1**; 536/24.3; 536/24.32; 536/24.33; 435/6; 435/91.2; 435/320.1; 435/240.2; 435/254.11; 435/254.2; 435/172.3; 435/69.1; 530/300; 530/350

[58] **Field of Search** 435/6, 91.2, 320.1, 435/240.2, 254.11, 254.2, 172.3, 69.1; 536/23.1, 24.3, 24.33, 265; 530/300, 350

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Primary Examiner—W. Gary Jones

Assistant Examiner—Dianne Rees

Attorney, Agent, or Firm—Townsend and Townsend and Crew LLP

[57] **ABSTRACT**

Methods and compositions are provided for the production of a polypeptide which is immunologically cross-reactive with a naturally-occurring major outer membrane protein (MOMP) of *Chlamydia trachomatis*. A DNA construct including a replication system recognized by *E. coli*, and an MOMP gene under the transcriptional control of a β-galactosidase promoter and terminator is provided. Recombinant phage λgt11/L2/33 was deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852, on Jan. 10, 1985 and granted accession no. 40157. L2 B9-F DNA was deposited at the American Type Culture Collection on Dec. 31, 1985, and granted accession No. 40217.

13 Claims, 8 Drawing Sheets

The approximately 1.1 kb insert DNA was sequenced by standard techniques, and the sequence is set forth in FIG. 1.

Sequencing of λ1059 Inserts

Lambda 1059 recombinants having 9.2 to 9.8 kb inserts that were shown to be homologous with λgt11/L2/33 by Southern analysis were used for endonuclease restriction mapping, and additional Southern analyses. Two contiguous fragments (BamHI/EcoRI and EcoRI/EcoRI) were identified, and these contain sufficient base pairs to encode for the L_2 MOMP gene product. These fragments were cloned into M13 for DNA sequencing. The sequence data for a 9.2 kb fragment (designated L2 B9-F DNA) are set forth in FIG. 2.

The sequence includes an untranslated region comprising 1287 bases, followed by a 66 base region encoding a 22 amino acid leader sequence. Coding for the MOMP begins at base 67 (amino acid 23) and extends through base number 1182 (amino acid 394). The molecular weight for the MOMP including the leader is calculated to be 42,557 daltons.

The N-terminus of the MOMP was located on the basis of the 25 amino acid N-terminus reported by Nano et al. (1985) supra. Differences in the sequences of the N-terminus reported by Nano et al. and that reported herein are found at amino acid residues 32, 44, and 45, as numbered in FIG. 2. These differences may result from differences among the isolates or mistakes in amino acid sequencing.

The sequence set forth in FIG. 1 corresponds to amino acids 247 through the 3'-terminus in FIG. 2, with certain deviations. Bases 36–38 in FIG. 1 are AGA, corresponding to amino acids GlyGlu, while bases 773–775 in FIG. 2 are TGT, corresponding to amino acids GlyVal. These deviations are underlined in both Figures. The DNA sequence corresponding to amino acids 305 through 394 in FIG. 2 has several deviations from FIG. 1 which result in a different reading frame for the sequence of FIG. 2. Base numbers 174, 181, and 186 in FIG. 1 were not detected in the λ 1059 clones. Base number 35 in FIG. 1 is a T, while the corresponding base in FIG. 2 (in amino acid 357) is a C. Finally, a G is inserted in amino acid 358 and a G is inserted in amino acid 374 in the sequence of FIG. 2. In both FIGS. 1 and 2, bases which are inserted or changed relative to the other Figures are boxed, while deleted bases are indicated by an arrow. Both the DNA and amino acid sequences of FIG. 2 are believed to be correct.

According to the subject invention, novel recombinant DNA constructs are provided for the expression of a polypeptide having immunological activity corresponding to that of a naturally-occurring major outer membrane protein of *Chlamydia trachomatis*. Such polypeptides may find use as reagents in the detection of *Chlamydia trachomatis* or antibodies to *Chlamydia trachomatis*, and as vaccines against infection by *Chlamydia trachomatis* in susceptible hosts.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

What is claimed is:

1. A DNA construct comprising a first DNA sequence segment encoding a polypeptide of at least 12 amino acids of the *Chlamydia trachomatis* major outer membrane protein (MOMP), operably linked to additional DNA sequence segments required for the expression of said first DNA sequence segment.

2. The DNA construct of claim 1, wherein the MOMP polypeptide encoded by the first DNA segment is from *C. trachomatis* serovar L2.

3. An isolated polynucleotide encoding a polypeptide of at least 12 amino acids of the *Chlamydia trachomatis* major outer membrane protein (MOMP).

4. The isolated polynucleotide of claim 3, wherein the MOMP polypeptide encoded thereby is from *C. trachomatis* serovar L2.

5. The isolated polynucleotide molecule of claim 3 encoding a *C. trachomatis* MOMP polypeptide, the sequence of said polynucleotide molecule comprising a coding strand for a MOMP polypeptide having an amino acid sequence as shown in FIG. 1 or FIG. 2.

6. The isolated polynucleotide molecule of claim 3 encoding a *C. trachomatis* MOMP polypeptide, the sequence of said polynucleotide molecule comprising a coding strand for a serovar variant of the MOMP polypeptide having an amino acid sequence as shown in FIG. 1 or FIG. 2.

7. A cultured cell line which expresses the *C. trachomatis* MOMP polypeptide encoded by the DNA construct of claim 1.

8. The cultured cell line of claim 7, which is eukaryotic.

9. The cultured cell line of claim 8, which is mammalian.

10. A method for producing a *C. trachomatis* MOMP polypeptide, comprising the steps of culturing the cell line of claim 7 and expressing the *C. trachomatis* MOMP polypeptide.

11. The method of claim 10, further comprising the step of purifying the *C. trachomatis* MOMP polypeptide which is expressed.

12. The DNA construct of claim 2, wherein the MOMP polypeptide encoded by the first DNA segment is from *C. trachomatis* serovar L2 as shown in FIG. 1 or FIG. 2.

13. The isolated polynucleotide of claim 2, wherein the MOMP polypeptide encoded thereby is from *C. trachomatis* serovar L2 as shown in FIG. 1 or FIG. 2.

* * * * *